Figure 1:

```
                                                                  Score     E
Sequences producing significant alignments:                       (bits)  Value

gi|1168223|sp|P35563|5HT3_RAT  5-hydroxytryptamine 3 recepto...    96    8e-19
gi|13242306|ref|NP_077370.1|  (NM_024394) 5-Hydroxytryptamin...    96    1e-18
gi|9938026|ref|NP_064670.1|  (NM_020274) 5-hydroxytryptamine...    94    2e-18
gi|2144046|pir||I58179 5HT3 receptor subunit - rat (fragmen...    94    3e-18
gi|4504543|ref|NP_000860.1|  (NM_000869) 5-hydroxytryptamine...    93    5e-18
gi|13325275|gb|AAH04453.1|AAH04453  (BC004453) Unknown (prot...    93    5e-18
gi|9790622|gb|AAB37533.2|  (S82612) 5-hydroxytryptamine type...    93    5e-18
gi|1586341|prf||2203408A  serotonin 3AS receptor [Homo sapiens]    93    7e-18
gi|11559956|ref|NP_071525.1|  (NM_022189) 5-hydroxytryptamin...    92    1e-17
gi|5174469|ref|NP_006019.1|  (NM_006028) 5-hydroxytryptamine...    92    2e-17


Top Alignment

>gi|1168223|sp|P35563|5HT3_RAT       5-hydroxytryptamine 3 receptor precursor (5-HT-3)
(Serotonin-gated
          ion channel receptor) (5-HT3R)
 gi|681916|dbj|BAA08388.1|   (D49395) serotonin 5-HT3 receptor [Rattus rattus]
         Length = 483

 Score = 95.9 bits (237), Expect = 8e-19
 Identities = 81/335 (24%), Positives = 157/335 (46%), Gaps = 28/335 (8%)

Query: 12  LGFSITLLLVHGQG-----FQGTAAIWPSLFNVN--LSKKVQESIQIPNNGSAPLLVDVR 64
           L   +++L+  G+G      Q  +   P+L  ++  L    ++ ++   +   P LV +
Sbjct: 10  LALFLSVLIAQGEGSRRRATQAHSTTQPALLRLSDHLLANYKKGVRPVRDWRKPTLVSID 69

Query: 65  VFVSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTSAHPR-HAITLPWESLWTPRLTILE 123
           V +  + NVD     +++ +  R  W D  L W           +++P +S+W P + I E
Sbjct: 70  VIMYAILNVDEKNQVLTTYIWYRQFWTDEFLQWTPEDFDNVTKLSIPTDSIWVPDILINE 129

Query: 124 ALWVDWRDQSPQARVDQDGHVKLNLALATETNCNFELLHFPRDHSNCSLSFYALSNTAME 183
           + V      P   V   G V+    L   T C+ ++ +FP D  NCSL+F +  +T  +
Sbjct: 130 FVDVGKSPSIPYVYVHHQGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTFTSWLHTIQD 189

Query: 184 LEF-------------QAHVVN----EIVSVKREYVVYDLKTQVPPQQLVPCFQVTLRLKN 227
           +                ++  +N    E++ V  ++  + ++T     ++   F V +R +
Sbjct: 190 INISLWRTPEEVRSDKSIFINQGEWELLGVFTKFQEFSIETSNSYAEMK--FYVVIRRR- 246

Query: 228 TALKSIIALLVPAEALLLADVCGGLLPLRAIERIGYKVTLLLSYLVLHSSLVQALPSSSS 287
             L   ++LL+P+  L++ D+ G  LP  + ER+ +K+TLLL Y V    +   LP+++
Sbjct: 247 -PLFYAVSLLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDTLPATAI 305

Query: 288 CNPLLIYYFTILLLLLFLSTIETVLLAGLLARGNL 322
           PL+  YF + + LL +S  ET+ +  L+ + +L
Sbjct: 306 GTPLIGVYFVVCMALLVISLAETIFIVQLVHKQDL 340
```

Figure 2:

```
PSSMs producing significant alignments: Score(bits) Evalue

gnl|CDD|4842 pfam02931, Neur_chan_LBD, Neurotransmitter-gated ion-channel l... 54.5 6e-09


            CD-Length = 216 residues, only  64.4% aligned
            Score = 54.5 bits (131), Expect = 6e-09

Query:  52   PNNGSAPLLVDVRVFVSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTSAHP-RHAITLP  110
Sbjct:  19   VRNGGDPVVVSVGLYLQQIISVDEKNQDLTTNVWLRQQWTDPRLAWNPSDYGGITSLRLP  78

Query:  111  WESLWTPRLTILEALWVDWRDQSPQ----ARVDQDGHVKLNLALATETNCNFELLHFPRD  166
Sbjct:  79   SDKIWKPDIFLYNR--ADGIHDITTPNTNVRVYPDGTVLWSPPAIYKSSCPMDLTYFPFD  136

Query:  167  HSNCSLSFYALSNTAMELEFQ  187
Sbjct:  137  QQNCSLKFGSWTYNGDEVDLQ  157
```

Figure 3:

Transmembrane Regions:

Helix 1 from 230 (out) to 253 (in) : Score = 5.26
Helix 2 from 261 (in) to 278 (out) : Score = 3.31
Helix 3 from 295 (out) to 314 (in) : Score = 4.96
Helix 4 from 368 (in) to 390 (out) : Score = 5.47
4 helices (-) : Score = 24.541

Masked Sequence: >INPIONCH1

MALWSLLHLTFLGFSITLLLVHGQGFQGTAAIWPSLFNVNLSKKVQESIQIPNNGSAPLL
VDVRVFVSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTSAHPRHAITLPWESLWTPRLT
ILEALWVDWRDQSPQARVDQDGHVKLNLALATETNCNFELLHFPRDHSNCSLSFYALSNT
AMELEFQAHVVNEIVSVKREYVVYDLKTQVPPQQLVPCFQVTLRLKNTALKSIIALLVPA
EALLLADVCGGLLPLRAIERIGYKVTLLLSYLVLHSSLVQALPSSSSCNPLLIYYFTILL
LLLFLSTIETVLLAGLLARGNLGAKSGPSPAPRGEQREHGNPGPHPAEEPSRGVKGSQRS
WPETADRIFFLVYVVGVLCTQFVFAGIWMWAACKSDAAPGEAAPHGRRPRL

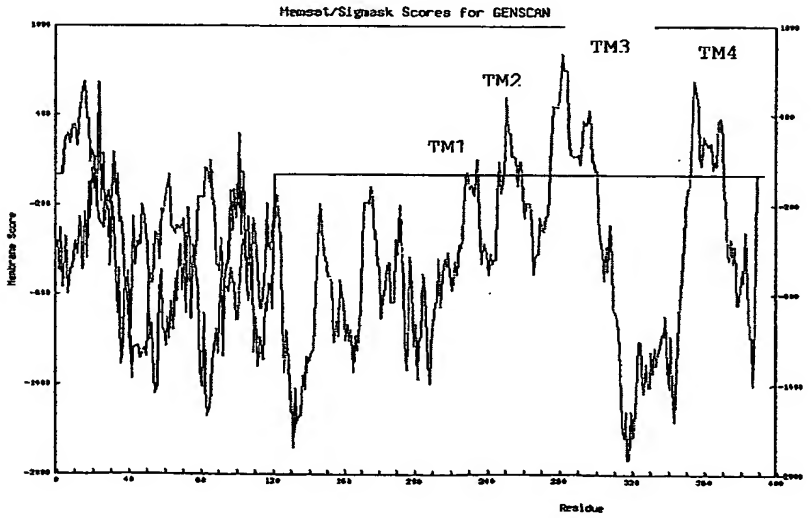


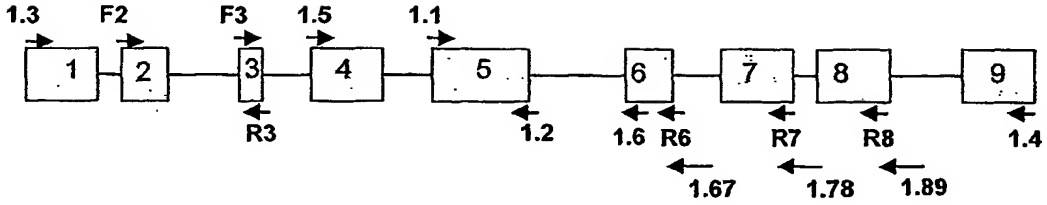
Figure 4:

```
5HT3B_MOUSE      --------MILLWSCLLVAVVGILGTATPQ---PGNSSLHRLTRQLLQQYHKEVRPVYNW
5ht3B_RAT        --------MILLWSCLLVAVVGILGTATPQ---PGNSSLHRLTRQLLQQYHKEVRPVYNW
5HT3B_HUMAN      ---MLSSVMAPLWACILVAAG-ILATDTHH---PQDSALYHLSKQLLQKYHKEVRPVYNW
5HT3_RAT         MPLCIPQVLLALFLSVLIAQGEGSRRRATQAHSTTQPALLRLSDHLLANYKKGVRPVRDW
5HT3_MOUSE       MRLCIPQVLLALFLSMLTAPGEGSRRRATQED-TTQPALLRLSDHLLANYKKGVRPVRDW
5HT3_HUMAN       MLLWVQQALLALLLPTLLAQGEARRSRN-----TTRPALLRLSDYLLTNYRKGVRPVRDW
5HT3C_HUMAN      MEGGWPARQSALLCLTVSLLLQGRGDAFTINCSGFDQHGVDPAVFQAVFDRKAFRPFTNY
INPIONCH1        --MALWSLLHLTFLGFSITLLLVHGQGFQG---TAAIWPSLFNVNLSKKVQESIQIPNNG
                                                          :: .:   :

5HT3B_MOUSE      AEATTVYLDLCVHAVLDVDVQNQKLKTSVWYREVWNDEFLSWNSSLFDEIQEISLPLSAL
5ht3B_RAT        AEATTVYLDLCVHAVLDVDVQNQKLKTSMWYREVWNDEFLSWNSSLFDDIQEISLPLSAI
5HT3B_HUMAN      TKATTVYLDLFVHAILDVDAENQILKTSVWYQEVWNDEFLSWNSSMFDEIREISLPLSAI
5HT3_RAT         RKPTLVSIDVIMYAILNVDEKNQVLTTYIWYRQFWTDEFLQWTPEDFDNVTKLSIPTDSI
5HT3_MOUSE       RKPTTVSIDVIMYAILNVDEKNQVLTTYIWYRQYWTDEFLQWTPEDFDNVTKLSIPTDSI
5HT3_HUMAN       RKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPEDFDNITKLSIPTDSI
5HT3C_HUMAN      SIPTRVNISFTLSAILGVDAQLQLLTSFLWMDLVWDNPFINWNPKECVGINKLTVLAENL
INPIONCH1        SAPLLVDVRVFVSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTS-AHPRHAITLPWESL
                   . * : . :  ::.**     :.: :     * :  : *...      :::  . :

5HT3B_MOUSE      WAPDIIINEFVDVERSPDLPYVYVNSSGTIRNHKPIQVVSACSLQTYAFPFDIQNCSLTF
5ht3B_RAT        WAPDIIINEFVDVERSPDLPYVYVNSSGTIRNHKPIQVVSACSLQTYAFPFDIQNCSLTF
5HT3B_HUMAN      WAPDIIINEFVDIERYPDLPYVYVNSSGTIENYKPIQVVSACSLETYAFPFDVQNCSLTF
5HT3_RAT         WVPDILINEFVDVGKSPSIPYVYVHHQGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTF
5HT3_MOUSE       WVPDILINEFVDVGKSPNIPYVYVHHRGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTF
5HT3_HUMAN       WVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTACSLDIYNFPFDVQNCSLTF
5HT3C_HUMAN      WLPDIFIVESMDVDQTPSGLTAYISSEGRIKYDKPMRVTSICKLDIFYFPFDQQNCTFTF
INPIONCH1        WTPRLTILEALWVDWRDQSPQARVDQDGHVKLNLALATETNCNFELLHFPRDHSNCSLSF
                 * * : * * : :    .   . :   * :.   .:   : *.::   ** * .**:::*
```

```
5HT3B_MOUSE      NSILHTVEDIDLGFLRNREDIEND-KRAFMNDSEWQLLSVSSTYHIRQS-SAGDFAQIRF
5ht3B_RAT        NSILHTVEDIDLGFLRNQEDIEND-KRSFLNDSEWQLLSVTSTYHIRQS-SAGDFAQIRF
5HT3B_HUMAN      KSILHTVEDVDLAFLRSPEDIQHD-KKAFLNDSEWELLSVSSTYSILQS-SAGGFAQIQF
5HT3_RAT         TSWLHTIQDINISLWRTPEEVRSD-KSIFINQGEWELLGVFTKFQEFSIETSNSYAEMKF
5HT3_MOUSE       TSWLHTIQDINITLWRSPEEVRSD-KSIFINQGEWELLEVFPQFKEFSIDISNSYAEMKF
5HT3_HUMAN       TSWLHTIQDINISLWRLPEKVKSD-RSVFMNQGEWELLGVLPYFREFSMESSNYYAEMKF
5HT3C_HUMAN      SSFLYTVDSMLLGMDKEVWEITDTSRKVIQTQGEWELLGIN-KATPKMSMGNNLYDQIMF
INPIONCH1        YALSNTAMELEFQAHVVN--------EIVSVKREYVVYDLKTQVPPQQL-----VPCFQV
                   :   *  .: :                .  . *: :  :                 : .

5HT3B_MOUSE      NVVIRRCPLAYVVSLLIPSIFLMLVDLGSFYLPPNCRARIVFKTNVLVGYTVFRVNMSDE
5ht3B_RAT        NVVIRRCPLAYVVSLLIPSIFLMLVDLGSFYLPPNCRARIVFKTNVLVGYTVFRVNMSDE
5HT3B_HUMAN      NVVMRRHPLVYVVSLLIPSIFLMLVDLGSFYLPPNCRARIVFKTSVLVGYTVFRVNMSNQ
5HT3_RAT         YVVIRRRPLFYAVSLLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDT
5HT3_MOUSE       YVIIRRRPLFYAVSLLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDT
5HT3_HUMAN       YVVIRRRPLFYVVSLLLPSIFLMVMDIVGFYLPPNSGERVSFKITLLLGYSVFLIIVSDT
5HT3C_HUMAN      YVAIRRRPSLYIINLLVPSSFLVAIDALSFYLPAESENRAPFKITLLLGYNVFLLMMNDL
INPIONCH1        TLRLKNTALKSIIALLVPAEALLLADVCGGLLPLRAIERIGYKVTLLLSYLVLHSSLVQA
                   : ::. .    : **:*:  *:  *  .  **  .  *  :* .:*:.* *:   : :

5HT3B_MOUSE      VPRSAGCTPLIGVFFTVCMALLVLSLSKSILLIKFLYEE----------RHSGQERP--L
5ht3B_RAT        VPRSAGCTSLIGVFFTVCMALLVLSLSKSILLIKFLYEE----------RHSEQERP--L
5HT3B_HUMAN      VPRSVGSTPLIGHFFTICMAFLVLSLAKSIVLVKFLHDE----------QRGGQEQP--F
5HT3_RAT         LPATAIGTPLIGVYFVVCMALLVISLAETIFIVQLVHKQDLQRPVPDWLRHLVLDRIAWL
5HT3_MOUSE       LPAT-IGTPLIGVYFVVCMALLVISLAETIFIVRLVHKQDLQRPVPDWLRHLVLDRIAWI
5HT3_HUMAN       LPATAIGTPLIGVYFVVCMALLVISLAETIFIVRLVHKQDLQQPVPAWLRHLVLERIAWL
5HT3C_HUMAN      LPAS--GTPLISVYFALCLSLMVVSLLETVFITYLLHVATT--------QPPPMPRWLHS
INPIONCH1        LPSSSSCNPLLIYYFTILLLLLFLSTIETVLLAGLLARG----------------------
                   :* :   ..*:  :*.: : ::.:*  :::.:  ::
```

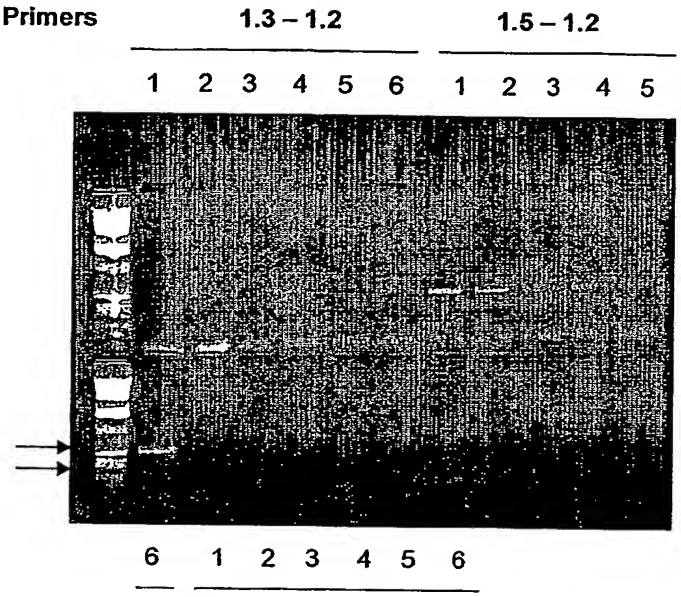
```
5HT3B_MOUSE      MCLQGDSDAEESRLYLGAPR----------------------ADVTESPVHQEHRVPSD
5ht3B_RAT        MCLRGDSDANESRLYLRAPC----------------------AEDTESPVRQEHQVPSD
5HT3B_HUMAN      LCLRGDTDADRPRVEPRAQR----------------------AVVTESSLYGEHLAQPG
5HT3_RAT         LCLGEQPMAHRPPATFQANKTDDCS------AMGNHCSHVGSPQDLEKTSRSRDSPLPPP
5HT3_MOUSE       LCLGEQPMAHRPPATFQANKTDDCSGSDLLPAMGNHCSHVGGPQDLEKTPRGRGSPLPPP
5HT3_HUMAN       LCLREQSTSQRPPATSQATKTDDCS------AMGNHCSHMGGPQDFEKSPRDRCSPPPPP
5HT3C_HUMAN      LLLHCTSPGRCCPTAPQKGN----------------------KGLGLTLTHLPGPKEPG
INPIONCH1       -NLGAKSGPSPAPRG------------------------------EQREHGNPGPHPAE

5HT3B_MOUSE      -----TLKDFWFQFRSINNSLRTRDQIHQKEVEWLAILYRFDQLLFRIYLAVLGLYTVTL
5ht3B_RAT        -----TLKDFWFQLQSINNSLRTRDQVYQKEVEWLAILCHFDQLLFRIYLAVLGLYTVTL
5HT3B_HUMAN      -----TLKEVWSQLQSISNYLQTQDQTDQQEAEWLVLLSRFDRLLFQSYLFMLGIYTITL
5HT3_RAT         REASLAVRGLLQELSSIRHSLEKRDEMREVARDWLRVGYVLDRLLFRIYLLAVLAYSITL
5HT3_MOUSE       REASLAVRGLLQELSSIRHFLEKRDEMREVARDWLRVGYVLDRLLFRIYLLAVLAYSITL
5HT3_HUMAN       REASLAVCGLLQELSSIRQFLEKRDEIREVARDWLRVGSVLDKLLFHIYLLAVLAYSITL
5HT3C_HUMAN      -----ELAGKKLGPRETEPDGGSAWTKTQLMELWVQFSHAMDTLLFRLYLLFMASSILTV
INPIONCH1        -------EPSRGVKGSQRSWPETADRIFFLVYVVGVLCTQFVFAGIWMWAACKSDAAPGE

5HT3B_MOUSE      CSLWALWSRM
5ht3B_RAT        CSLWALWSRM
5HT3B_HUMAN      CSLWALWGGV
5HT3_RAT         VTLWSIWHYS
5HT3_MOUSE       VTLWSIWHYS
5HT3_HUMAN       VMLWSIWQYA
5HT3C_HUMAN      IVLWNT----
INPIONCH1       AAPHGRRPRL
```

Figure 5:



IC1.1	GCTCTGGGTGGACTGGAGGG
IC1.2	CCGTGTTGCTGAGAGCGTAGAAGC
IC1.3	CCGGAATTCATGGCCCTATGGTCCCTGCT
IC1.4	CCCAAGCTTTTACAGTCTAGGCCGCCTGC
IC1.5	GACACTCGCCTGGCCTGGAACACTA
IC1.78	GCAACCCACTGCTCATTTACTACTT
IC1.R3	CAGCCTAAGCAGCAGCATGG
IC1. R7	TGAGCAGTGGGTTGCAGGAG
IC1.F2	TCTGGCCATCCCTCTTCAAC
IC1.F3	GACATCCTGCGATACACAAT

Figure 6:



1 – adrenal
2 – heart
3 – prostate
4 – skeletal muscle
5 – testis
6 – thymus

Figure 7:

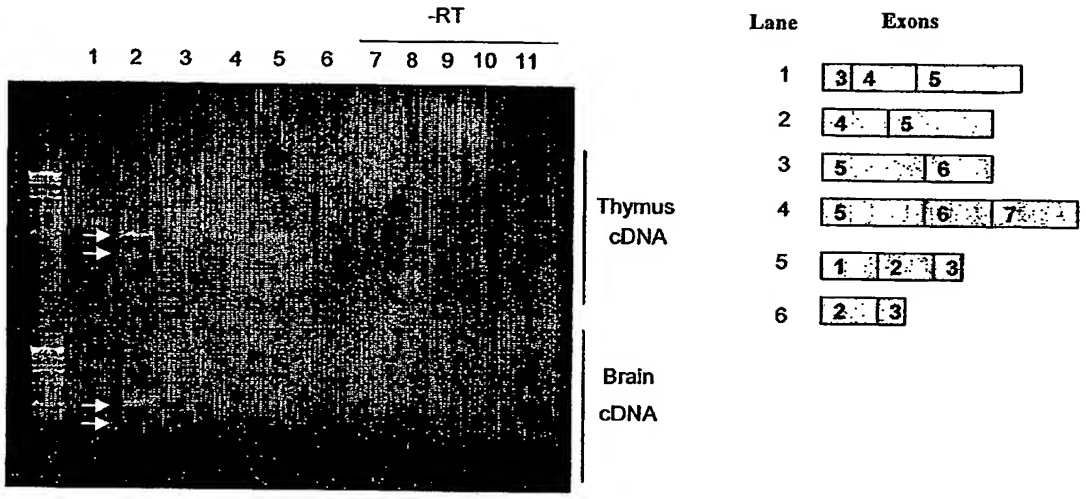

-RT
1 2 3 4 5 6 7 8 9 10 11
Thymus cDNA
Brain cDNA
Lane
Exons
1
3 4 5
2
4 5
3
5 6
4
5 6 7
5
1 2 3
6
2 3

Figure 8A:

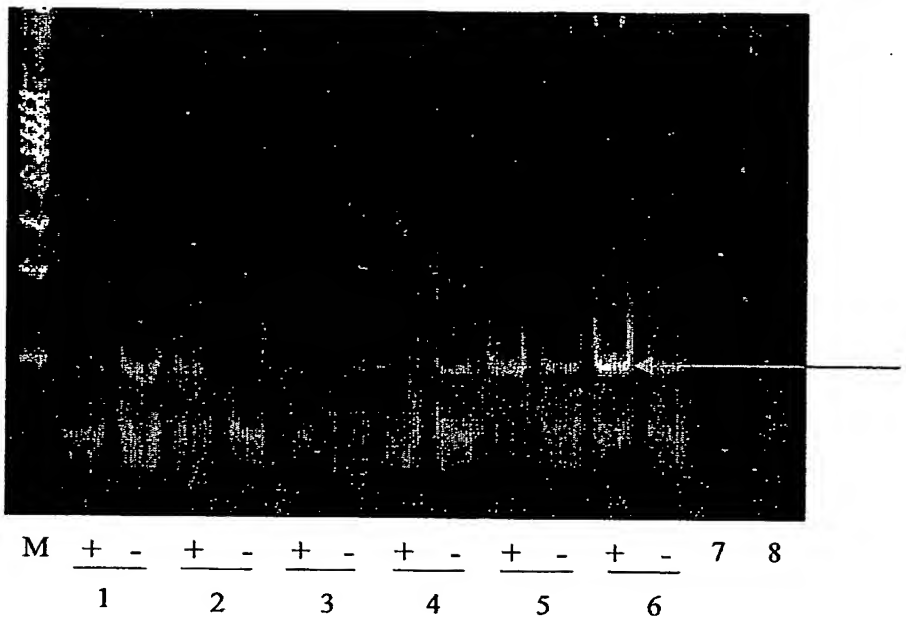


Figure 8B:

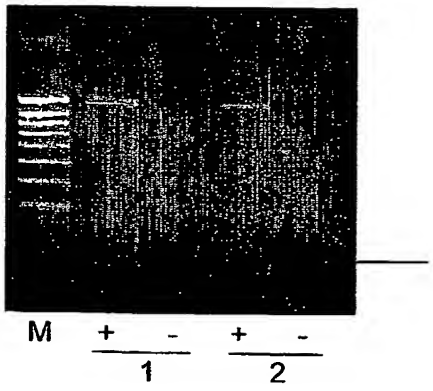
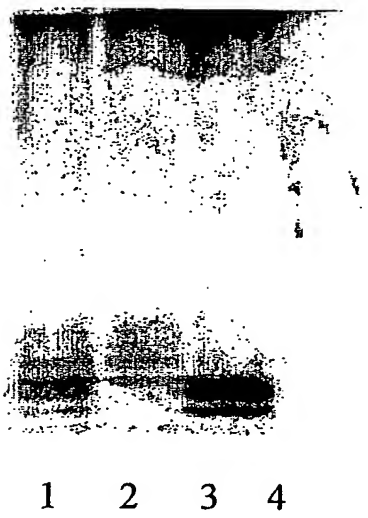
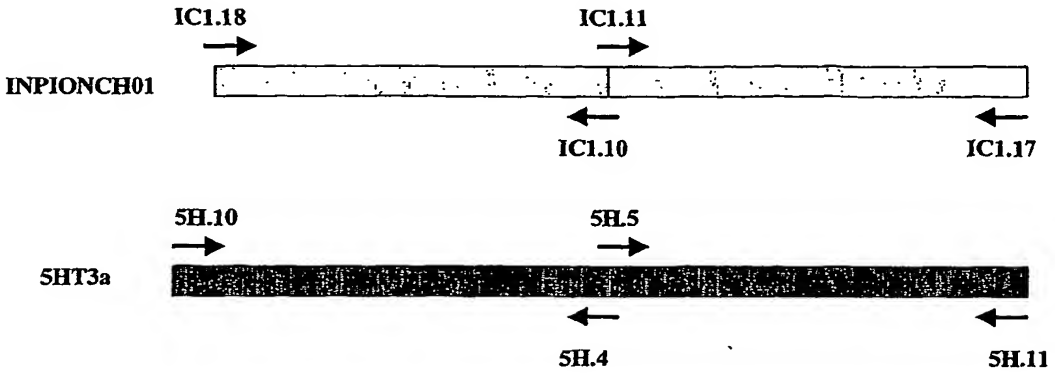


Figure 9:



1 - 5HT3a + INPIONCH01
2 - 5HT3a
3 - INPIONCH01
4 - untransfected cells

Figure 10:



IC1.10	CCGGAATTCCGCCGTGTTCTTCAGCCTCAG
IC1.11	CCGGAATTCCTCAAGTCCATCATCGCTCTC
IC1.17	CTAGTCTAGACTCAGTCTAGGCCGCCTGCCAT
IC1.18	CCCAAGCTTGCCACCATGGCCCTATGGTCCCTGCT
5H.4	CCGGAATTCGCGGATGACCACATAGAACTTC
5H.5	CCGGAATTCCGGCGGCCCCTCTTCTATGTG
5H.10	CCCAAGCTTGCCACCATGCTGCTGTGGGTCCAGCA
5H.11	CTAGTCTAGACTAGCGTACTGCCAGATGGACCAGAGCAT

Figure 11:

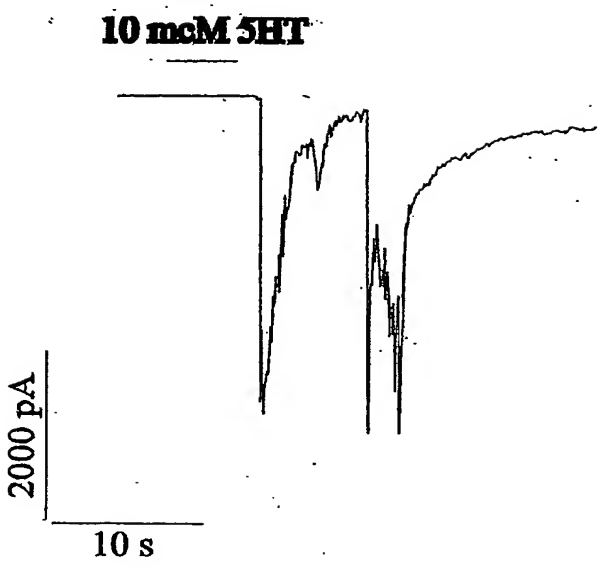


Figure 12:

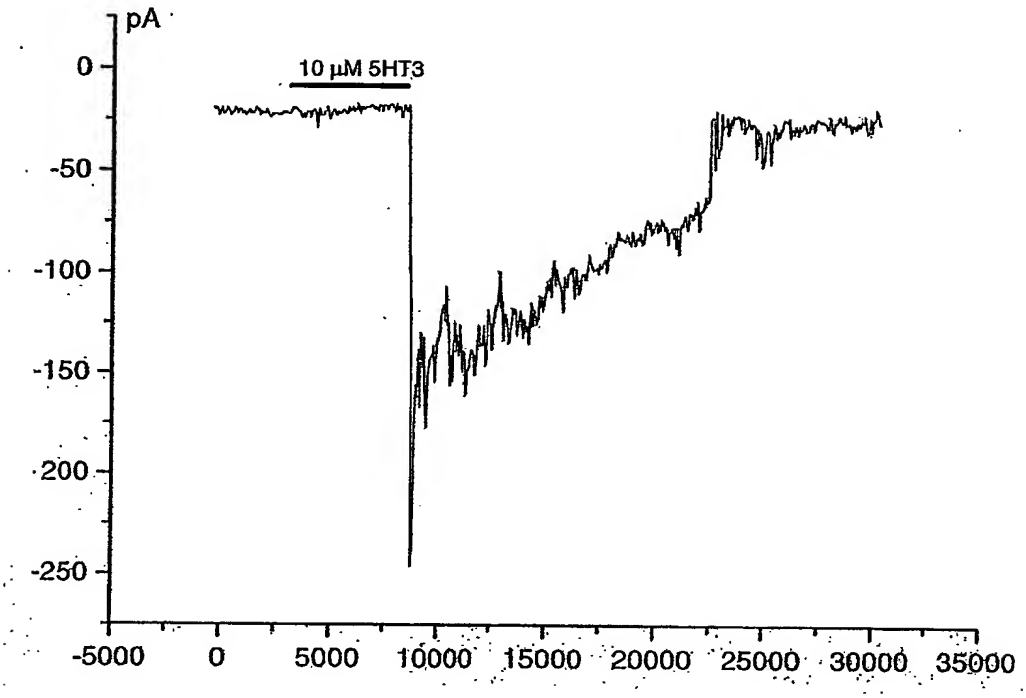
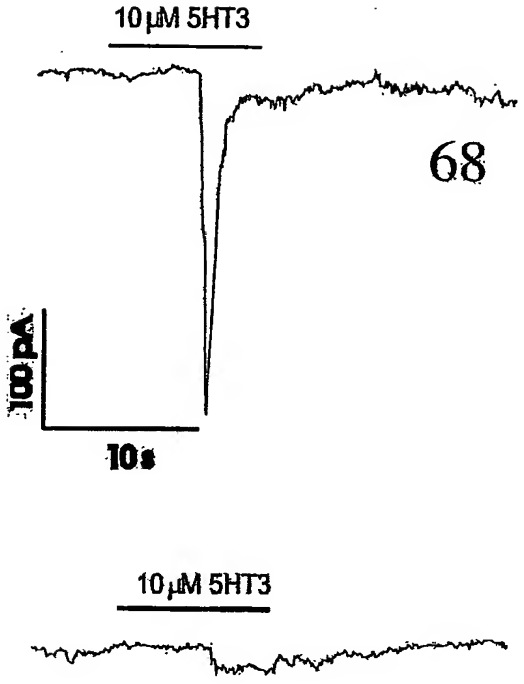

pA
0
-50
-100
-150
-200
-250
10 µM 5HT3
-5000
0
5000
10000
15000
20000
25000
30000
35000

Figure 13:



2nd application after wash

Figure 14:

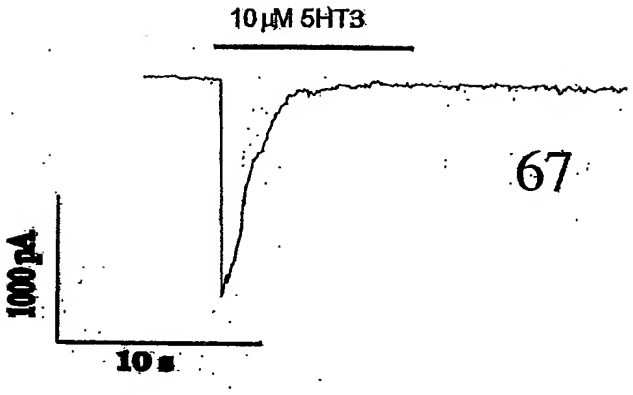
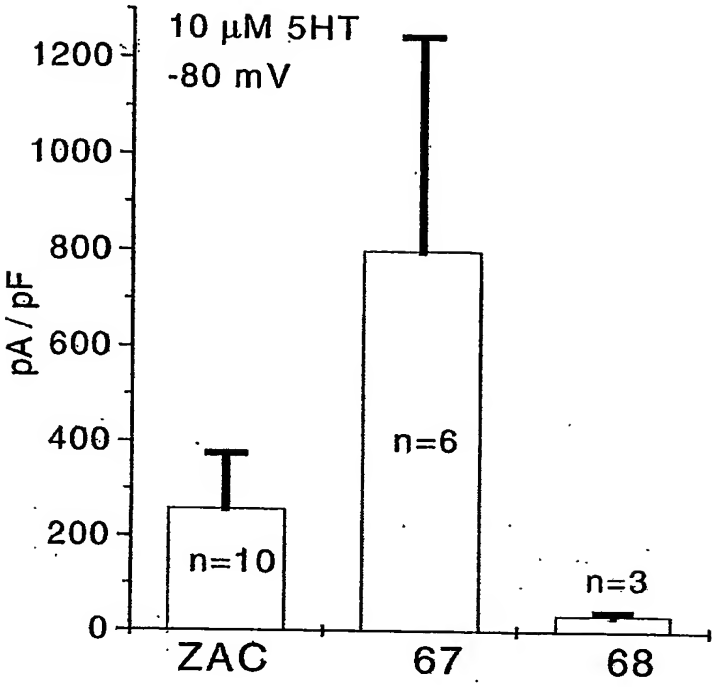


Figure 15:



Amplitude of serotonin-induced (10 mcM) currents.

FIG. 16

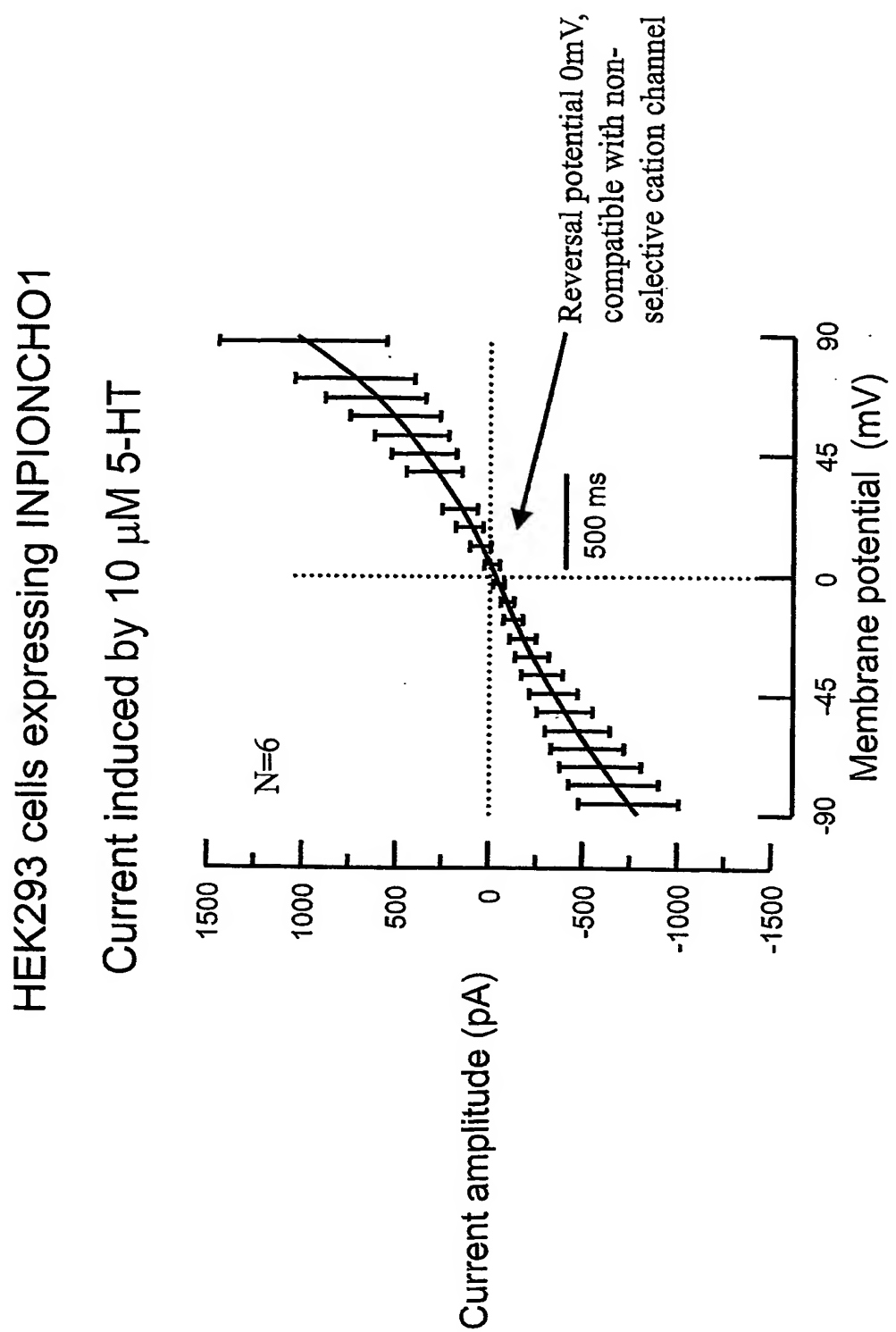

HEK293 cells expressing INPIONCHO1
Current induced by 10 µM 5-HT
N=6
Reversal potential 0mV, compatible with non-selective cation channel
500 ms
Current amplitude (pA)
1500
1000
500
0
-500
-1000
-1500
-90
-45
0
45
90
Membrane potential (mV)

Figure 17:

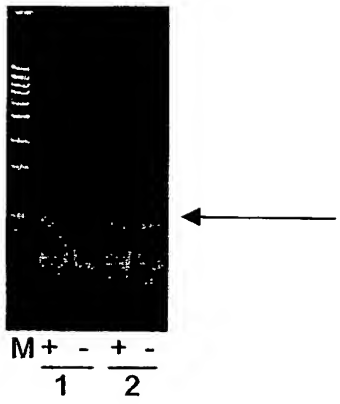


Figure 18: